Isolation of a nodulating, nitrogen-fixing bacterium of dune lupine

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Introduction

Figure 1. Dune lupine near the El Segundo Sand Dunes

Lupinus chamissonis (dune lupine) is a known nodulating species of the legume plant family found natively along the California coast. In the Ballona wetlands and El Segundo sand dunes near Loyola Marymount University, there is an ongoing effort to understand the effects of urban runoff on sand dunes, where dune lupine grows. In particular, this study investigates the symbiosis between *L. chamissonis* and nodulating, nitrogen-fixing rhizobia bacteria and how urban stresses, such as heavy metal contamination, can impact this symbiosis.

Figure 2. View of Ballona Wetlands in Los Angeles, CA

Materials and methods

Identification of nodulating bacteria

- Nodules from *L. chamissonis* roots were surface sterilized, crushed, bacteria isolated, and pure cultures made of individual isolates.
- *L. chamissonis* seeds were germinated axenically and then inoculated with isolated strains.
- Nodulation status was checked and bacteria were reisolated from nodules.
- The 16S rDNA gene was amplified by polymerase chain reaction (PCR) for sequencing analysis.

Results

Nodulation

At least two strains of bacteria were isolated from nodules, one fast-growing (LC4) and the other slow growing (LC2). The fast growing bacterium was identified as *Pantoea agglomerans*, although we were unable to obtain nodules when plants were reinoculated. We are still in the process of identifying the slow-growing rhizobia, which we had confirmed nodulates dune lupine, thus fulfilling Koch's postulates. We also found that a slow-growing strain of *Bradyrhizobium canariense*, originally isolated from *L. perennis* nodules, could nodulate dune lupine. We are in the process of confirming its identity.

Figure 3. Bacteria isolated from nodules of lupine plants in the wild were able to nodulate plants.

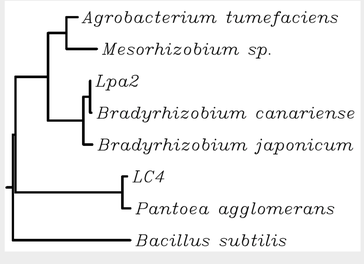

Figure 4. Phylogenetic tree showing that Lpa2 (isolated from *L. perennis* nodules) and LC4 (isolated from *L. chamissonis* nodules) cluster with *B. canariense* and *P. agglomerans* respectively.

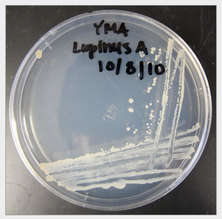

Figure 5. Bacterial sample Lpa-1 isolated from *L. chamissonis* roots grows slowly on YMA media.

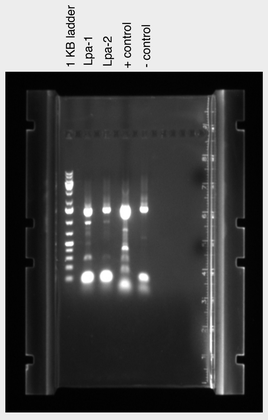

Figure 6. PCR products of 16S rDNA visualized by gel electrophoresis. From left to right: A) DNA ladder, B) bacterial isolate sample Lpa-1, C) bacterial isolate sample Lpa-2, D) positive control, E) negative control.

Conclusions

- A slow-growing strain of rhizobia was isolated and found to nodulate dune lupine. Although we are in the process of identifying it, we hypothesize that it is a species of *Bradyrhizobium* as this genus is often found to nodulate lupines.
- *B. canariense*, originally isolated from *L. perennis*, nodulates dune lupine.
- *P. agglomerans* was isolated from dune lupine nodules but does not cause the formation of nodules. *P. agglomerans* has previously been found in nodules of *Hedysarum*.

Decreasing numbers of *L. chamissonis* along its native California coast suggest that the species does not adapt well to the effects of urbanization. An increase of heavy metals due to urban runoff is likely to affect the growth of these plants, which provide ecosystem services to the near-extinct El Segundo Blue Butterfly, as well as other local wildlife. Continuing research on *L. chamissonis* and its nodulating symbiont is vital to ongoing restoration efforts of the Ballona wetlands.

Future work

Now that we have isolated a nitrogen-fixing bacterium of dune lupine, there is work to be done in identifying the isolate. To do this, we will perform PCR on the 16S ribosomal DNA and have it sequenced. The sequence will be compared to a database using BLAST and we will likely be able to determine what the species of the isolate is.

There is also much work left to be done on determining the role that heavy metal plays in the association. *L. chamissonis* plants will be treated with various concentrations of zinc sulfate, and then inoculated. Their growth and nodulation will then be observed.

Literature cited

Y. Benhizia, H. Benhizia, A. Benguedouar, et al. Gamma proteobacteria can nodulate legumes of the genus *Hedysarum*. System. Appl. Microbiol. 27: 462-468.

A. Jarabo-Lorenzo, R. Perez-Galdona, J. Donate-Correa et al. 2003. Genetic diversity of Bradyrhizobial populations from diverse geographic origins that nodulate *Lupinus* spp. and *Ornithopus* spp. Systematic and Appl. Microbiol. 26: 611-623.

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For further information

Please contact *mpina3@lion.lmu.edu*. More information on this and related projects can be obtained at *www.teamlum.com*. A PDF-version of the poster is also available.